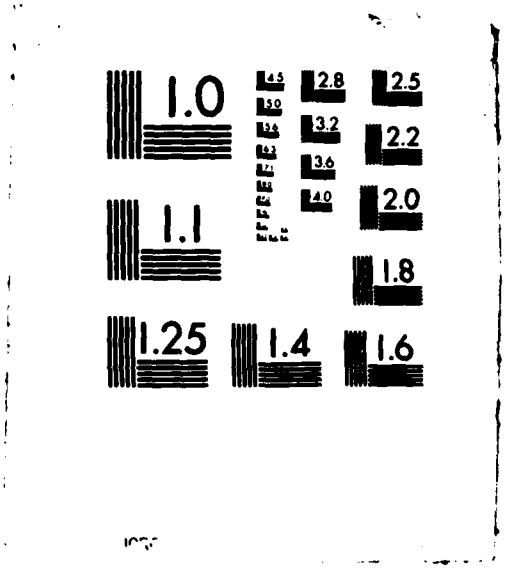
AD-A172 279 TENDON FIBROPLASIA INDUCTION BY EXOGENOUS ELECTRICAL FIELDS(U) MEDICAL COLL OF VIRGINIA RICHMOND DEPT OF PHYSIOLOGY AND BIOPHYSICS S F CLEARY ET AL. 10 AUG 86 1/1

UNCLASSIFIED N00014-84-K-0539 F/G 6/3 NL


1.0
1.1
1.25
1.4
1.6
1.8
2.0
2.2
2.5
2.8
3.2
3.6
4.0

AD-A172 279

DISTRIBUTION STATEMENT A
Approved for public release!
Distribution Unlimited

(2)

SECURITY CLASSIFICATION OF THIS PAGE

DTIC ELECTE FEB 0 6 1986 S D

REPORT DOCUMENTATION PAGE

1a. REPORT SECURITY CLASSIFICATION (U)	1b. RESTRICTIVE MARKINGS N/A
2a. SECURITY CLASSIFICATION AUTHORITY N/A	3. DISTRIBUTION/AVAILABILITY OF REPORT Distribution Unlimited
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE N/A	
4. PERFORMING ORGANIZATION REPORT NUMBER(S) N/A	5. MONITORING ORGANIZATION REPORT NUMBER(S)

6a. NAME OF PERFORMING ORGANIZATION Virginia Commonwealth Univ.	6b. OFFICE SYMBOL *(If applicable)* N/a	7a. NAME OF MONITORING ORGANIZATION Office of Naval Research	
6c. ADDRESS *(City, State, and ZIP Code)* Dept. of Physiology and Biophysics Medical College of Virginia Richmond, Virginia 23298		7b. ADDRESS *(City, State, and ZIP Code)* 800 North Quincy Street Arlington, Virginia 22217-5000	
8a. NAME OF FUNDING/SPONSORING ORGANIZATION Office of Naval Research	8b. OFFICE SYMBOL *(If applicable)* ONR	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER N00014-84-K-0539	
8c. ADDRESS *(City, State, and ZIP Code)* 800 North Quincy Street Arlington, Virginia 22217-5000		10. SOURCE OF FUNDING NUMBERS	
		PROGRAM ELEMENT NO. 61153N	PROJECT NO. RR04108
		TASK NO. NR or 441	WORK UNIT ACCESSION NO

11 TITLE *(Include Security Classification)*
Tendon Fibroplasia Induction by Exogenous Electrical Fields

12 PERSONAL AUTHOR(S)
Cleary, Stephen F.; Liu, Li-Ming.

13a. TYPE OF REPORT Annual	13b. TIME COVERED FROM 8/1/85 TO 7/31/86	14. DATE OF REPORT *(Year, Month, Day)* 86-08-10	15. PAGE COUNT 7

16. SUPPLEMENTARY NOTATION
N/A

17. COSATI CODES			18. SUBJECT TERMS *(Continue on reverse if necessary and identify by block number)*
FIELD	GROUP	SUB-GROUP	
08			

19. ABSTRACT *(Continue on reverse if necessary and identify by block number)*

A parametric study is being undertaken of the effects of extra low frequency (ELF) conductive electric fields on chicken tendon explant fibroplasia, collagen synthesis and oriented migration. Independent variables are: pulse repetition rate, pulse duration, peak current density (or electric field strength), explant orientation (with respect to E field) and donor (chicken) age. ELF pulsed electric fields with average current densities in the range of 0.18 to 5.7 $\mu A/cm^2$ induced statistically significant alternations in fibroblast proliferation with a windowed response of peak stimulation of proliferation in the range of 0.4 to 1 $\mu A/cm^2$, and suppression at higher current densities.

DTIC FILE COPY

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT ☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT ☐ DTIC USERS	21 ABSTRACT SECURITY CLASSIFICATION (U)
22a NAME OF RESPONSIBLE INDIVIDUAL Dr. T. C. Rozzell	22b. TELEPHONE *(Include Area Code)* 202/696-4053
	22c. OFFICE SYMBOL ONR

DD FORM 1473, 84 MAR 83 APR edition may be used until exhausted. All other editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE

Annual Scientific Report

ONR Contract Number N00014-84-K-0539

Title: Tendon Fibroplasia Induction by Exogenous Electrical Fields

Investigators: Stephen F. Cleary, Li-Ming Liu and Robert Diegelmann
Departments of Physiology and Biophysics and Plastic Surgery
Medical College of Virginia, Richmond, VA 23298

Report Period: August 1, 1985 - July 31, 1986

The primary objective of this contract has been to develop a tissue model-system to enable us to investigate the relationship between exposure to weak ELF electric fields and fibroplastic events in tendon in vitro. The research was directed to satisfy four basic criteria:

a. the fibroblastic cells (tenoblasts) should be within their native tissue matrix,

b. cells should be exposed to ELF fields in an environment that simulates as closely as possible the in vivo situation,

c. cell proliferation, migration and collagen and protein synthesis-the principal fibroplastic events-should be studied under identical conditions, thus permitting a determination of the interrelationship between these events, and finally,

d. all of the physical parameters should be accurately known and controlled such that the probability of artifacts could be minimized such as, for example, as a result of Joulian heating of the tendon explants during exposure.

These basic criteria have been satisfied during the first two years of study, resulting in the development of a tendon explant model system that has been used to quantitatively determine the effects of ELF electric field exposure.

1) Tendon Explant Model System

Procedures were developed for the culture of explants obtained from flexor digitorum profundus tendons obtained from the long digits of female white Leghorn chickens. Explants are cultured in DMEM supplemented with 1% or 10% fetal calf serum and 25mM Tricine buffer (pH 7.4) under sterile conditions at 37°C in a tissue culture incubator.

The initial phase of the study involved exploring various means of minimizing the explant-to-explant variations in proliferation and migration. It was determined that the primary sources of variation were the matrix used to attach the explant to the culture dish and the anatomical source of the explant. Experiments conducted using different matrix materials indicated that uniform fibroblast migration could be obtained from tendon explants during the first 8 days of culture using either a fibrin matrix or a matrix consisting of agar and fibrin. Since the fibrin matrix consistently resulted in a significantly enhanced fibroblastic proliferative response relative to the agar-fibrin matrix, and since the fibrin more closely satisfied study criteria (b), fibrin has been used in all subsequent experiments.

The rate of proliferation and migratory patterns (to a lesser extent) of explants obtained from various flexor tendon anatomical locations differed due to variations in the relative amounts of connective tissue components (viz. mesotenon, vinculum, epitenon, endotenon and tendon bundle) in different parts

of the tendon. Although complete surgical removal of mesotenon reduced the sample-to-sample variation in proliferation, differences were still evident between explants taken from the sublimis or profundus regions of flexor digitorum tendons. Consistently uniform growth was obtained using explants from the distal segment of the profundus tendon which has been subsequently used as the source of explants.

2) Fibroblast Migration

Fibroblast migration patterns from tendon explants have been studied by phase contrast microscopy at various times during culture. Although visual and photomicroscopic observations suggested that ELF electric fields may affect cell migration by inducing polarization relative to the agar salt-bridge electrodes, it has been difficult to document this phenomenon, especially during the latter phases of explant culture. After approximately 8 days of culture, retraction of the fibrin clot occurs as a result of tensile forces between the proliferating fibroblasts and the fibrin matrix. Retraction, which occurs in both ELF exposed and sham-exposed cultures at approximately the ninth day of culture, obscures the effects of ELF fields on fibroblast migration. By conducting studies of cell migration earlier during the culture interval and altering the size of the fibrin matrix, we have reduced perturbations caused by clot retraction. Clot retraction places a limit of 9-10 days on the duration of explant exposure to ELF fields.

To facilitate study of the effect of electric field polarity on fibroblast proliferation and migration, 4- and 6-mm tendon explant sections have been used in addition to the 2mm plug explants used in our earlier

studies. Explants were initially prepared by the use of trephines, but recently developed apparatus for cutting tendons using a specially designed sectioning chamber and scalpel blades, now provides greater uniformity.

The effect of field polarity on explant fibroblast migration from 4- and 6-mm explant sections has been investigated by orienting the longitudinal axis of the explants parallel or perpendicular to the electric field. Parallel alignment appears to induce a relatively greater amount of proliferation at the end of the explant facing the anode and a greater overall rate of proliferation than perpendicularly oriented explants. In the case of explants aligned perpendicular to the field lines, there appears to be preferential migration perpendicular to the field lines (i.e. parallel to the longitudinal explant axis). Field-induced fibroblast polarization has not been a consistent, reproducible effect, which is attributed in part, at least, to inherent variability between explants and within explants. Results of our earlier studies, as well as studies of others, suggest that polarized migration of fibroblasts (and other cells) can be more reproducibly induced by electric fields of significantly higher field strength or current density than used in our current studies. We have evidence, however, that fibroblast proliferation may be suppressed under such conditions.

3) Assay Methods

Assay methods have been developed for the simultaneous determination of fibroblast proliferation and collagen synthesis in explant cultures using ^{3}H-thymidine and ^{14}C-proline incorporation. This double-label technique has enabled us to determine the relative rates of cell proliferation and collagen, noncollagen protein, and total protein synthesis during 24 hour pulse

labelling periods at any time during ELF or sham-exposure of tendon explants. This assay method has been used to determine the effects of ELF parameters on proliferation and protein synthesis in 5 and 8 day explant cultures.

4) Exposure System

A thermostated temperature-controlled circulation chamber enables us to simultaneously expose (or sham expose) 8 explant cultures to a wide range of current densities with precise (ie. ± 0.2°C) and accurate (ie. ± 0.1°C) temperature control. The use of this system of temperature control (as well as theoretical knowledge of the rate of dissipation of electrical energy in the cultures) provides assurance that field-induced effects are not a consequence of temperature variations in the exposed samples. Agar-culture media salt bridges permit electric fields to be delivered to the explant cultures in the absence of any electrolytic products. To eliminate any possibility of electrolysis products contaminating the cultures, the agar salt bridges are replaced daily.

5) Fibroblast Proliferation and Collagen Synthesis

The majority of the ELF exposures were conducted using the following ranges of parameters:

- unipolar (positive) square wave, pulse repetition rate 1, 5, or 16.6-Hz
- current density: average 0.2-5.7 $\mu A/cm^2$
  peak 142-355 $\mu A/cm^2$

- electric field strength: average 12-335 μV/cm
  peak 8.4-21 mV/cm
- specific absorption rate (SAR): average 2.4-1910 pW/g
  maximum 1.2-7.4 μW/g
- pulse duration: 1, 5ms
- temperature: 37 ± 0.2°C
- exposure duration: 5 to 9 days (24h per day)

Experiments were conducted to determine the effects of variation of ELF pulsed field-parameters, as well as effects of explant donor age, explant size, and orientation relative to the electric field polarity. Although the data are not yet extensive enough to permit conclusions to be drawn regarding the relationship between ELF field parameters and tendon explant fibroplasia and collagen synthesis, the following trends have been noted:

a) Considering the relationship of fibroblast proliferation to the average current density, there is an enhancement in the range of approximately 0.4-1 $\mu A/cm^2$. The maximum average increase, which occurred at 0.71 $\mu A/cm^2$ is on the order of 40%, whereas the maximum absolute % difference was 86%.

b) Statistical analyses (Student's t test) indicate that the majority of the results that show a statistically significant ($p < 0.05$) enhancement in proliferation are in the current density range of 0.36 to 0.71 $\mu A/cm^2$.

c) Statistically significant inhibition of proliferation occurred at the high current density limit (ie. 5.7 $\mu A/cm^2$).

d) Limited data on the effects of pulse duration suggest maximum stimulation with 1 ms pulses relative to 5 ms pulses.

e) Again, based on limited data, the maximally effective pulse repetition rate appears to be 1-Hz (relative to 5- or 16-Hz), although this may be due to differences in time-averaged current density per se, rather than pulse repetition rate (the same reasoning may apply to (d) above).

f) Polarization effects are suggested in that proliferation was enhanced when the explants were oriented parallel to electric field lines and suppression occurred for perpendicular orientation at a current density of 1.8 $\mu A/cm^2$.

g) No statistically significant effects on ^{14}C-proline incorporation were detected. Additional studies using a bacterial collagenase assay are necessary to determine the effects of ELF electric fields on collagen synthesis.

h) There is an inverse relationship between explant donor age and fibroblast proliferation, with no interaction with ELF apparent in the presently available data.

In summary, ELF pulsed electric fields with average current densities in the range of 0.18 to 5.7 $\mu A/cm^2$ induce statistically significant alterations in the rates of fibroblast proliferation in chicken tendon explants in vitro. Proliferation shows a "windowed" type response with a peak in the 0.4-1 $\mu A/cm^2$ average current range. Data does not permit firm conclusions to be drawn regarding the effect of pulse duration and/or pulse repetition rate, but the maximally effective combination (with respect to induction of proliferation) has been 1m duration, 1-Hz pulses.

SC/ms/4800/CLEAR7

END

10-86

DTIC